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Amino Acid Sequence of the Light Chain from a Mouse Myeloma Protein with Anti-Hapten Activity: Evidence for a Third Type of Light Chain

(anti-2,4-dinitrophenyl activity/carboxyl-terminal residues/N-terminal residues/κ and λ chains)

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ABSTRACT **A tentative amino acid sequence has been determined for the light chain of a mouse A-myeloma (MOPC-315) protein with anti-2,4-dinitrophenyl activity. An unusual amino acid sequence in the carboxyl-terminal 104 residues suggests that the chain represents a new class of immunoglobulin light chain. The "variable" segment of this chain (amino-terminal 110 residues) differs at only eight positions from the corresponding segment of the lambda chain of a mouse M-myeloma (MOPC-104) protein with anti-dextran activity.**

The recent finding of myeloma proteins with antibody activity (e.g., 1–3) and the development of procedures for the preparation of conventional antibodies of remarkably restricted heterogeneity (4, 5) provide opportunities to correlate the primary structure of immunoglobulins and their specific reactivities with particular haptens and antigens. Accordingly, we are studying the structure of protein 315, an A-myeloma protein that binds 2,4-dinitrophenyl (Dnp) ligands with high specificity (6). In this paper, we present a tentative amino acid sequence for the light chain (L^{315}) of this protein. An unusual sequence suggests that this chain represents a new type, resembling immunoglobulin light chains of the λ type more than those of the κ type.

Protein 315 was isolated from sera of mice bearing transplants of MOPC-315, a plasma cell tumor*, whose origin is described below. Fully reduced carboxymethylated or aminoethylated light chains were prepared as described (40), and digested with trypsin, thermolysin, or pepsin. The peptides were purified by ion-exchange chromatography on Dowex resins (7) or SE-Sephadex (8), and their amino acid sequences were determined by Edman degradation (9). The successively removed amino-terminal residues were identified by: subtractive analysis, gas-liquid chromatography of CH_3CNS amino acids (9), the dansyl procedure (10), or combinations of these methods. Fragments obtained by cyanogen bromide treatment of L^{315} were purified by gel filtration on Sephadex G-100 in 5 M guanidine·HCl, followed by chromatography on CM-cellulose in 8 M urea (11). Antisera to L^{315} were prepared by immunizing rabbits with the Fab fragment of protein 315 in complete Freund's adjuvant, and absorbing the sera with κ- and λ-containing A-myeloma proteins that were produced by plasma cell tumors MOPC-460 and S-176*.

A complete set of peptides was not isolated from any one enzymatic digest, but a combination of peptides from trypsin and thermolysin digests accounted for the amino acid composition of the chain (Table 1), and provided the basis for the provisional sequence shown in Fig. 1. As few overlaps have been established thus far, the alignment of peptides was based largely on homology with the complete amino acid sequence of the mouse λ light chain that is produced by plasma cell tumor MOPC-104 (12); this light chain is hereafter referred to as L^{104}. There are two methionine residues in L^{315}, and the three purified cyanogen bromide fragments obtained from this chain (corresponding to the N-terminal 87 residues, the central 88 residues, and the C-terminal 39

TABLE 1. *Amino acid composition of mouse light chains**

	315	104	41
Lys	10	9	11
His	5	5	2
Arg	5	6	9
Asp	16	15	23
Thr	29	30	18
Ser	22	24	38
Glu	19	21	20
Pro	12	11	9
Gly	19	17	13
Ala	15	15	9
Cys/2	5	5	5
Val	16	17	9
Met	2	2	2
Ile	6	5	10
Leu	16	15	16
Tyr	2	7	8
Phe	11	6	7
Trp	4	5	4
total	214	215	213

* The compositions for L^{104} and L^{41} are based on published amino acid sequence data (12, 18). The composition of L^{315} is based on the sequence in Fig. 1 (compare ref. †). L^{41} is a κ chain; L^{104} and L^{315} are λ1 and λ2 chains (see *text*).

Abbreviations: Nomenclature of immunoglobulins and their chains and fragments follows the recommendations of the World Health Organization (*Bull. W.H.O.*, **30**, 447 (1964). Myeloma proteins are designated by numbers and the tumors that produce them by preceding letters; e.g., tumor MOPC-315 produces 315.

* MOPC-315, MOPC-460, and S-176 have been maintained by serial subcutaneous transplantation in BALB/c mice. These plasma-cell tumors were generously provided by M. Potter of the National Cancer Institutes (MOPC-315 and MOPC-460), and by M. Cohn and M. Weigert of the Salk Institute (S-176).

† Underdown, B. J., E. S. Simms, and H. N. Eisen, *Biochemistry* in press (1971).

TENTATIVE SEQUENCE OF L-315

LIGHT CHAIN OF THE IgA FROM MOPC-315

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  1  PCA ALA/VAL VAL THR GLX GLX SER ALA LEU THR THR SER PRO GLY GLY THR/THR VAL/LEU
 21  THR[CYS]ARG)SER SER THR GLY ALA VAL THR THR SER ASN TYR* ALA ASN TRP ILE GLX GLX
 41  PRO(ASX LYS HIS LEU PHE THR GLY)LEU(ILE GLY GLY THR SER ASX)ARG ALA PRO GLY VAL
 61  PRO VAL ARG/PHE SER GLY SER(LEU ILE GLY ASX)LYS/ALA ALA/LEU THR/ILE THR GLY(ALA
 81  GLX THR GLX ASX GLX ALA(MET)TYR PHE[CYS]ALA LEU TRP PHE ARG/ASX HIS PHE VAL PHE
101  GLY GLY GLY THR LYS/VAL(THR VAL LEU GLY GLN PRO)LYS SER THR PRO THR LEU THR VAL
121  PHE PRO PRO SER SER GLX GLX LEU LYS GLU ASN LYS/ALA THR LEU VAL[CYS]LEU ILE SER
141  ASX PHE(SER PRO GLY SER VAL THR)VAL ALA TRP LYS/ALA ASX(GLY THR PRO)ILE THR GLX
161  GLY(VAL ASX THR THR ASX PRO SER)LYS GLX GLY ASX LYS/PHE(MET)ALA SER SER(PHE LEU
181  HIS)LEU THR ASX(SER TRP GLX)ARG/SER HIS ASX PHE SER THR[CYS]GLN VAL THR ASX(GLX
201  GLY HIS THR)VAL GLX LYS SER LEU SER PRO ALA GLU[CYS]LEU
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FIG. 1. The tentative amino acid sequence of L^{315}, the light chain of the A-myeloma protein produced by MOPC-315. / indicates that overlaps between peptides have not been established. Residues within parenthesis have been determined by composition and are aligned by homology to the sequence of L^{104} (12). * The tyrosine at position 34 has been labeled specifically with two different affinity-labeling reagents (15, 17). The two methionines and five cysteine residues are enclosed by circles and boxes.

residues) have compositions that are consistent with the alignment shown.

L^{315} has five cysteine residues in the same positions at which they occur in all other light chains (13). Its amino-terminus is blocked (pyrrolidone carboxyl residue), as is characteristic of λ chains (13), but it is the only immunoglobulin chain described so far, in any species, with a C-terminal leucine (14, 15). Other evidence (obtained early in this study) that L^{315} represents a novel type of chain was provided by the isolation of the C-terminal octapeptide and nonapeptide from trypsin and pepsin digests, respectively. These peptides differed in three out of nine residues from the corresponding sequence in L^{104}, a mouse λ chain, and in five out of nine residues from mouse *k* chains (16). In addition, there are only two tyrosine residues in the entire L^{315} chain, and one was clearly shown by the affinity-labeling studies of Goetzl and Metzger (15), and of Haimovich *et al.* (17) to be in the N-terminal half of the chain. This allowed one tyrosine at most for the C-terminal half of L^{315}, whereas four tyrosines are present in this segment of mouse *k* and λ chains (18, 12). As is shown in Fig. 2, L^{315} resembles mouse λ chains (73% identical residues) more closely than mouse *k* (40% identity) in the C-terminal 104 positions.

The following additional evidence also shows that L^{315} resembles λ more than *k*. (*a*) In its C-terminal 104 residues, L^{315} is more like human λ (65% identical residues) than human *k* (35% identical residues). (*b*) Alignment to achieve maximum homology with mouse *k* chains requires the same gaps in the C-terminal half of L^{315} as in the corresponding segments of mouse and human λ chains (Fig. 2). (*c*) The V segment (N-terminal 110 residues) of L^{315} differs from that of L^{104} by only eight residues, whereas it differs from the V segment of a mouse *k* chain (MOPC-41) by 62 residues. The small difference between the V segments of L^{315} and L^{104} is discussed below.

Are L^{315} and L^{104} allotypic variants of mouse λ chains? The genetic background of the mouse in which MOPC-315 arose is relevant to this question (ref. 19, 20). This mouse was in the seventh generation of successive backcrosses to inbred BALB/c mice, starting with the F_1 progeny of a BALB/c × C57BL/6 cross. The progeny selected for each backcross with BALB/c had both BALB/c and C57BL/6 allotypic heavy-chain markers on their immunglobulins (19). Genes for light and heavy chains are unlinked in man and rabbit (21, 22). If they are also unlinked in the mouse then, after seven backcross generations in which heavy chains were selected for, the probability that L^{315} is specified by a C57 BL/6 gene is 1/256 (i.e., $1/2^7 \times 1/2$, because only one of the alleles for an immunoglobulin light chain is expressed per cell). Hence, L^{315} is probably the product of a BALB/c gene. As L^{104} is also the product of a BALB/c gene, the two chains are not likely to be allotypic variants in this highly inbred mouse strain.

The following serological evidence also argues against an allelic relation between L^{315} and L^{104}. After absorption with two A-myeloma proteins, 460 (to remove antibodies to α chains) and 176 [to remove antibodies that cross-react with λ chains of the L^{104} type (12, 23)], rabbit antisera to 315 still reacted with L^{315} in a radioimmunoassay. (In this assay, the absorbed rabbit anti-L^{315} reacted with [^{125}I]-L^{315}, and the radioactive immune complexes were precipitated with goat antiserum specific for the Fc fragment of rabbit IgG.) The same amount of serum from normal BALB/c and C57BL/6 mice was required to inhibit this reaction and indicated that <0.1% of the immunoglobulins in each of these strains have 315-like light chains. However, the full significance of these results is not clear, as it is not yet known whether the anti-L^{315} serum is directed to the N-terminal or C-terminal half of L^{315}.

On the basis of the multiple residue differences in the C-terminal halves of L^{315} and L^{104}, and the serological evidence available, we believe that L^{315} represents a third type of light chain (i.e., an isotype) present in all mouse strains. We propose that light chains of the L^{315} type (assuming that

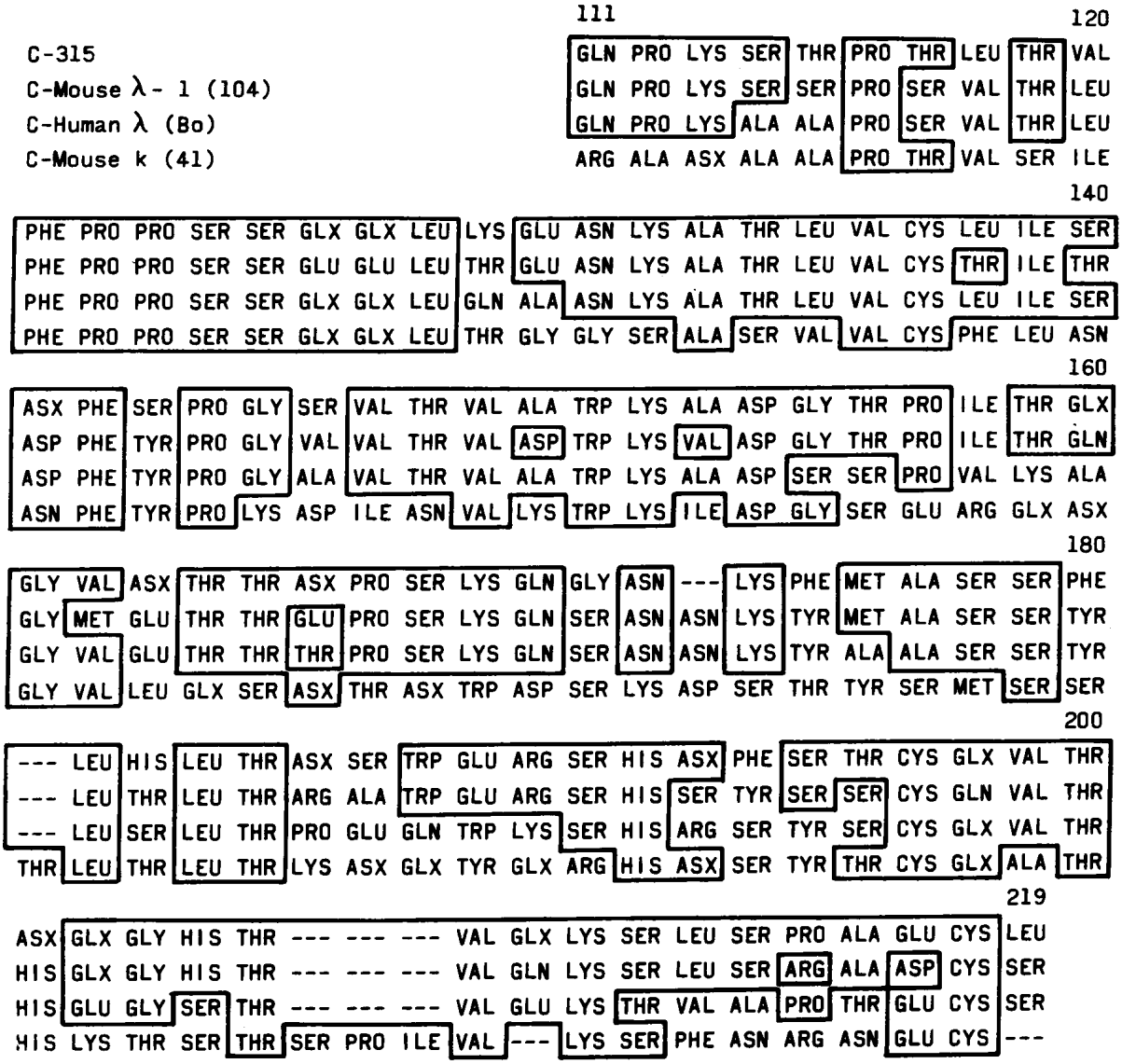

FIG. 2. Comparison of constant regions of light chains of 315, mouse λ (104), human λ (Bo), and mouse *k* (41) (refs. 12, 39, and 18, respectively). Positions at which L^{315} is identical to one or more of the other chains are enclosed. Position numbers are based on Gln at 111 in L^{104} and L^{315}. Gaps are introduced to achieve maximum homology.

others exist) be designated λ2, and that the more frequently encountered λ chain [represented by L^{104} and others (23)] be designated λ1. From the preliminary serological evidence cited above, it seems that λ2 chains cannot be present in more than a very small proportion of mouse immunoglobulins. The ε chain of IgE (present in normal human serum at 1/25,000 of the concentration of IgG) provides a striking example of a rarely expressed isotype (24).

Human immunoglobulins also have two types of λ chains, Oz^+ and Oz^- (25). As they have been found in all of 10 individuals so far tested, these forms of λ seem to be isotypes rather than allotypic variants (26). However, unlike mouse λ1 and λ2, which differ in 27% of residues in the C-terminal half of the chain, Oz^+ and Oz^- differ by only a single residue in this region.

Amino acid sequences of many light chains have clearly shown that human *k* chains resemble mouse *k* more than human λ (27). This suggests that genes for *k* and λ diverged before separate evolutionary lines of descent arose for man and mouse. In contrast, mouse λ1 and λ2 sequences resemble each other more than either resembles human λ (Fig. 2), and the close similarity of Oz^+ and Oz^- in man is even more striking. It seems likely, therefore, that the probable duplication of the λ gene that led to λ1 and λ2 occurred after the divergence of evolutionary lines for man and mouse. The duplication would most likely have occurred more recently in the line of human than in that of mouse descent.

A considerable body of evidence suggests that the same set of genes for the variable segments of heavy chains (V_H) can cooperate with any of several closely linked genes for constant segments (C_H) in specifying complete heavy chains (γ, μ, etc.) (28–34). A similar situation probably exists for λ1 and λ2, because the amino acid sequence in the V segment of L^{315} resembles so closely that of L^{104} and other λ chains (12, 23); thus, V_λ can be associated with $C_{\lambda 1}$ or $C_{\lambda 2}$. This also supports the view that L^{315} belongs to the λ family.

The few differences between the V segments of L^{315} and L^{104} are in accord with the unusually small degree of sequence variation in the N-terminal segments of mouse λ chains (12, 23). Table 2 lists the differences and their positions. Half of

TABLE 2. *Position at which L^{315} and L^{104} differ in the N-terminal 110 residues*

Position no.	315	104
16	Gly	Glu
38	Ile	Val
54	Ser	Asn
62	Val	Ala
87	Met	Ile
94	Phe	Tyr
95	Arg	Ser
98	Phe	Trp

the differences are located in regions that are particularly susceptible to sequence variation in light and heavy chains (35, 36). Three of the eight differences between L^{315} and L^{104} are clustered in a small region just beyond the cysteine residue at position 90; this region is generally subject to most variation. The only amino acid substitution that would require a 2-base change in nucleotide triplets also falls in this region (Phe-Trp, at position 98).

The residues listed in Table 2 probably contribute to the different ligand-binding specificities of protein 104 (anti-dextran) (37), and protein 315 (anti-Dnp). But it is not clear that these residues are actually contact amino acids for specifically bound ligands. In protein 315, Tyr_{34} of the light chain is affinity-labeled specifically by both a *m*-nitrobenzenediazonium salt (15) and by a bromoacetyl derivative of Dnp-ethylenediamine (17). Moreover, spectral evidence suggests that Trp_{37}, a highly conserved residue, might make contact with bound Dnp ligands (17). Possibly the variable residues (Table 2) determine the conformation of the site, permitting other residues, including some highly conserved ones, to make contact with bound ligands. It is also possible that some of the variable residues are intimately involved in or modulate the interaction between V_L and V_H regions, for there is also suggestive evidence for preferential pairing of certain light and heavy chains (38).

The small difference between V segments of L^{315} and L^{104}, and the marked differences in ligand-binding activities of proteins 315 and 104 (1, 6, 37), indicate that a few critical residues in the light chains have immense influence on the specificities of these macromolecules, or that their specificities are largely due to differences in their heavy chains. Sequence analysis of the 315 heavy chain is in progress.

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NOTE ADDED IN PROOF

Since this paper was submitted, additional nonallelic variants of the constant segment of human λ chains (C_λ) have been demonstrated by the finding of both Ser-Gly alternatives at position 153 in the light chains of all of 31 individuals tested [Gibson, D., M. Levanon, and O. Smithies, *Biochemistry*, **10**, 3117 (1971)]. We have also been informed by M. Potter, National Institutes of Health, that the mouse in which MOPC-315 arose was the product of successive BALB/c crosses with the C57BL/KA strain, not C57BL/6. This does not alter the sense of our arguments, though it obviously requires that a search for the 315-type of λ chain be made in the KA subline of C57BL mice.

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